BIOHYDROGEN PRODUCTION FROM PALM OIL MILL EFFLUENT USING POLYETHYLENE GLYCOL IMMOBILIZED CELLS IN UPFLOW ANAEROBIC SLUDGE BLANKET REACTOR

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ABSTRACT

The demand for improvement of the hydrogen production by dark hydrogen fermentation is increasing. Recently, a number of cell-immobilization systems were used to improve dark hydrogen production. The main objective of this research was to examine the polyethylene glycol immobilized cells system in enhancing hydrogen production and treatment of palm oil mill effluent (POME). During this research five experiments were performed. In the first experiment, PEG gel was fabricated and used as a carrier to immobilize *Clostridium sp.* for biohydrogen production using POME. POME was diluted and used as a substrate. The resulting PEG-immobilized cells were found to yield 5.35 L H_2/L-POME, and the maximum hydrogen production rate was 0.5 L H_2/L-POME/h (22.7 mmol/L h). The Monod-type kinetic model was used to describe the effect of substrate (POME) concentration on the hydrogen production rate. Furthermore, PEG- immobilized cell was examined for H_2 production in comparison to suspended cell reactor. The suspended-cell containing reactor was able to produce hydrogen at an optimal rate of 0.348 L H_2/L-POME/h at HRT 6 h. However, the immobilized-cell containing reactor exhibited better hydrogen production rate of 0.589 LH_2/ L-POME/h which occurred at HRT 2 h. When the immobilized-cell containing reactor was scaled up to 5 L, the hydrogen production rate was 0.553–0.589 L H_2/ L-POME/h. Another study addressed the application of a PEG-immobilized upflow anaerobic sludge blanket (UASB) reactor using *Clostridium sp.* for enhancing continuous hydrogen production from POME. The UASB reactor containing immobilized cells was operated at varying hydraulic retention times (HRT) that ranged from 24 to 6 h at 3.3 g chemical oxygen demand (COD)/L/h organic loading rate (OLR), or at OLRs that ranged from 1.6 to 6.6 at 12 h HRT. The maximum volumetric hydrogen production rate of 0.336 LH_2/L/h (15.0 mmol/L/h) with a hydrogen yield of 0.35 LH_2/g $COD_{removed}$ was obtained at a HRT of 12 h and an OLR of 5.0 g COD/L/h. The effect of immobilized cell packing ratio, HRT and POME concentration on continuous hydrogen production and treatment efficiency of palm oil mill effluent was studied. The UASB reactor with a PEG-immobilized cell packing ratio of 10% weight to volume ratio (w/v) was optimal for dark hydrogen production. The highest volumetric hydrogen production rate of 0.365 L H_2/L/h (16.2 mmol/L/h) with a hydrogen yield of 0.38 LH_2/g $COD_{removed}$ was obtained at POME concentration of 30 g COD/L and HRT of 16 h. The average hydrogen content of biogas and COD reduction were 68% and 66%, respectively. In the final study, optimization of the hydrogen production capability of the immobilized cells, including PEG concentration, cell loading, curing times as well as effects of temperature and different inorganic components concentrations on hydrogen production rate were studied. Result showed that with an optimal PEG concentration (10 % w/v), cell loading (2.4 g dry wt.), curing time (80 min) and inorganic components **(**$NiCl_2$ 1 mg/L, $FeCl_2$ 300 mg/L and $MgSO_4$ 100 mg/L), attaining an excellent hydrogen production rate of 7.3 L/L-POME/d and a hydrogen yield of 0.31 L H_2/g COD in continuous operation.

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CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

This chapter describes a brief description of palm oil industry in Malaysia, palm oil mill effluent (POME), cell immobilization, upflow anaerobic sludge blanket (UASB) reactor and hydrogen production. The first and second part of chapter explains about palm oil industry in Malaysia, hazard of POME wastewater and reuse of POME as fermentation media. Third part explain about cell immobilization system. The fourth part discusses production of biohydrogen. Finally, in the last part of this chapter, problem statement, objectives and scope of study are described.

1.2 BACKGROUND

1.2.1 Palm oil industry in Malaysia

Oil Palm was first introduced to Malaysia in year 1875 as an ornamental plant (DOE, 1999). Malaysia has the most ideal climate conditions for growing oil palm. The growth of the palm oil industry in Malaysia has been phenomenal over the last 4 decades. The Malaysian palm oil industry has grown rapidly over the past few years to become the world's second largest producer of palm oil, accounted for 10.3% of the world's oils and fats production in year 2007 (Lam and Lee, 2011). The palm oil industry served as an important backbone to the country economy and has significantly increased the standard of living among Malaysians (Yusoff and Hansen, 2007). Morever, the palm oil industry provides a source of livelihood to rural families in govermental land schemes and private small holders, as well as employment opportunities to, agricultural workers in estates (Wu et al., 2010). For example, in 2003, more than 3.79 million hectares of land were under oil palm cultivation, occupying more than one-third of the total cultivated area in Malaysia and 11% of the total land area. In year 2008, the total export of palm oil and derived products raked RM 64,808 million (USD 20,268 million), or 9.8% of the total national revenue (Yusof, and Yew, 2009). As of 2009, there were 416 palm oil mills operating in Malaysia, 249 mills from Peninsular Malaysia and 167 from Sabah and Sarawak. There were 120 mills with total capacity 29,893, 200 tonnes FFB per year located in Sabah alone. Total of 17,564,937 Metric Tons (MT) crude palm oil (CPO) produced in year 2009 and 31.03% of total CPO was produced in Sabah (MPOB, 2009). The oil palm planted area in 2011 increased 3% y/y to 5 mn hectares due to increase in planted area in Sabah and Sarawak. CPO production in 2011 increased 11.3% y/y to reach a record-high of 18.9 mn. It was claimed that further expansion of oil palm industry will cause severe negative impacts toward environment such as destruction of orangutan habitat, deforestation and green house gases (GHG) emission due to over exploration of peat land for oil palm plantation (Laurance et al., 2010, and Yule, 2010). Nevertheless, criticisms are not only limited to plantation sector, but also include palm oil mills.

1.2.2 The palm oil mill effluent

The production of palm oil is increasing every year due to its application for biodiesel production after the announcement of Fifth Fuel Policy under Eighth Malaysia Plan (2001–2005) (Lim and Teong, 2010). This leads to the increasing amount of palm oil mill effluent (POME); a by-products from the oil-palm extracting process (Poh and Chong, 2009). In Malaysia, the estimated annual production of palm oil mill effluent (POME) is about 50 million tons. POME is a viscous brown liquid cosisiting of 92-94% water, 6-7% total solids, 2-4% suspended solids (SS) and 0.7-0.8% oil. Discharge of untreated POME directly into the water streams, its is certain to cause considerable environmental problems due to highy value of chemical oxygen demands (COD) and biochemical oxygen demands

(BOD) that it generates (Lam and Lee, 2011). The palm oil industry in malaysia has thus been identified as that which discharges the largest pollution load into the water bodies and the environment throught the country (Wu et al., 2010). This adverse environmental effect from the palm oil industry cannot be ignored. Thus, there is an urgent need to find an efficient and practical approach to preserve the environment while maintaining the economy in good condition.

1.2.3 POME as fermentation media

The high compositions and concentrations of carbohydrate, protein, nitrogenous compound, lipid, mineral and nutrient content in POME makes it an ideal fermentation medium for biotechnological means (Hwang et al., 1978 and Habib et al., 1997). POME and its derivatives have been exploited as fermentation media to produce various products/metabolites such as bioinsecticides, antibiotics, polyhydroxyalkanoates, solvents, organic acids as well as enzymes to varying degrees of success (Wu, T.Y. 2007). Since POME contains high level of organic matters and thus, adoption of anaerobic digestion in the first stage of the treatment process is a necessity to convert the bulk of the wastes to biogas (biomethane). The treated effluent is further subjected to an aerobic treatment in order to meet the required discharge standards. These treatment steps have been applied either as an open pond or open digesting tank systems in Malaysian palm oil mills. However, due to the lack of infrastructure and low demand of renewable energy (biogas) in the county, biomethane is not captured but escapes directly to the atmosphere and thus caused serious air pollution. Methane has been categorized as one of the GHG with its global warming potential 21 times more potent than CO_2. On other hand, potential of using raw POME as the main substrate to produce biohydrogen has been intensively studied (Ismail et al., 2010) since the generated hydrogen and its combustion production do not count as green house gases (Koroneos et al., 2004). Naturally, POME contains lignocelluloses and hemicelluloses material (complex carbohydrate polymers) which resulted to high COD value (15,000–100,000 mg/L) (Chong et al., 2009a). Due to this reason, POME can become a suitable substrate for biohydrogen production and act as a wastewater treatment process simultaneously. To date, production of biohydrogen from POME in commercial scale is not ready based on current production technology.

1.2.4 Cell immobilization

An immobilized cell is defined as a microbe that prevented from moving independently of its neighbors to all parts of the aqueous phase of the system by natural or artificial means. Cells immobilization is a versatile tool that serves to increase the stability of a microbial system, allowing its application under extreme environmental conditions, its reuse and the development of continuous bioprocesses (Anisha and Prema, 2008). Immobilization of living cells can improve the process economy by increasing reusability and/or feasibility (Salter and Kell, 1991). This technology provides an innovative procedure for the immobilization of bacteria that can be used to improve the performance and stability of biological treatment systems. The use of immobilized cell systems is well documented for the production of valuable product like enzymes, antibiotics, organic acid and alcohols. Several methods can be applied to immobilization microorganism on the carriers by using artificial way, these methods including: covalent bonding, cross-linking of microorganism, adsorption and encapsulation into a polymer-gel and entrapment in a matrix, and so on (Cassidy et al., 1996). The cell entrapment is one of the most widely applied methods for cell immobilization, in which microorganisms are enclosed in a polymeric matrix which is porous enough to allow the diffusion of substrates to the cells and of product away from the cells. Entrapment of bacteria provides both an appropriate growth environment for the organism (Zhu et al., 1999) and structural, thermal and chemical stability to the entrapped cells (Dickson et al., 2009). Thus, immobilization-cell systems are also adapted with a feature of creating a local anaerobic environment, which is well suited to oxygen perceptive fermentative hydrogen production.

The application of immobilized cells to hydrogen production has been reported such as ethylene vinyl acetate copolymer (Wu et al., 2005b), polyvinyl alcohol (Tian et al., 2009), lignocellulosic materials (Kumar and Das, 2001), sodium alginate (Ishikawa et al., 2006), calcium alginate (Hu et al., 2007). Nearly all their work showed that immobilized fermentative bacteria can enhance and stabilize hydrogen production process. However, Natural polymers (agar, agarose, alginate, kappa-carragenan) possess poor mechanical strength and durability, although they are not toxic to microorganisms. Conversely, synthetic polymers have strong mechanical strength and durability but are often toxic to microorganisms (Kuu and Polack, 1983). Polyethylene glycol (PEG) is a promising type of synthetic polymer, which is cheap, non-toxic to microorganisms, good mechanical properties and highly porous structure that helps to sustain immobilized cell viability (Leenen et al., 1996). PEG is the only immobilization matrix used in large-scale wastewater–treatment plant in Japan and more than five years of gel durability has been demonstrated (Takeshima et al., 1993).

1.2.5 Up-flow anaerobic sludge blanket (UASB) reactor

In spite of their early introduction, the interest on anaerobic systems as the main biological step in wastewater treatment was scarce until the development of the upflow anaerobic sludge blanket (UASB) reactor in the early 70s (Lettinga et al., 1980). The success of the UASB concept depends on the establishment of a dense sludge bed in the bottom of the reactor, in which the biological processes take place (Lettinga, 1995). The formation of sludge bed takes place by the accumulation of the incoming suspended solids and the growth of bacteria. UASB reactor essentially consists of gas-liquid-solids separator (to retain the anaerobic sludge within the reactor), an influent distribution system and effluent draw off facilities. UASB reactor is a high effective, energy saving and less construction area technology. UASB process is a combination of physical and biological processes. The main feature of physical process is separation of solids and gases from the liquid and that of biological process is degradation of decomposable organic matter under anaerobic conditions. Wastewater enters at the bottom of the reactor. At the top, biogas is collected and the effluent of treated water leaves (Figure 1.1). At the upper part of the reactor, above the sludge bed, a blanket zone is formed where some particles of biomass are suspended. This zone acts as a separation zone between the water flowing up and the suspended biomass. Performance depends on the mean cell residence time and reactor volume depends on the hydraulic residence time, therefore, UASB reactor can efficiently convert wastewater organic compounds into biogas. Among thousands of anaerobic full scale tretment facilities worldwide, approximately 60% are based on the UASB design concept, treating a various range of industrial wastewaters (Jung et al., 2012 and Jantsch et al., 2002).

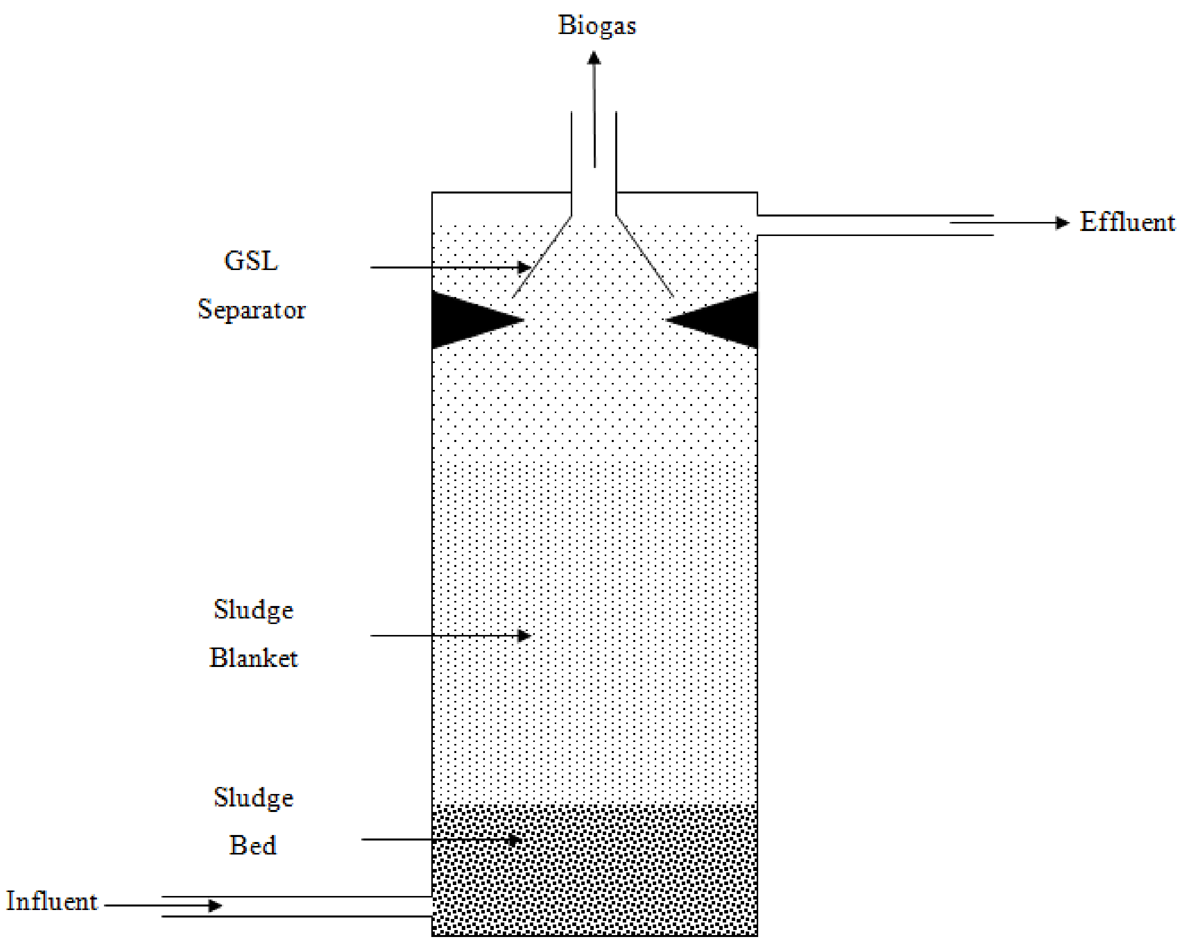

Figure 1.1: Upflow anaerobic sludge blanket reactor

1.2.6 Biohydrogen production

The joint challenges of environmental crises and dwindling fossil fuel supplies are driving intensive research focus in alternative energy production. Hydrogen is widely regarded as one of the most potential future energy vectors, capable of assisting in issues of environmental emissions, energy security and versatility as fuel (Pakarinen et al., 2008). When hydrogen is used as a fuel, its main combustion product is water which can be recycled again to produce more hydrogen. In recent years widely uses of hydrogen have been demonstrated *viz.* hydrogenfueled transit buses, ships and submarines etc., including chemical and petrochemical applications. Despite the “green” nature of hydrogen as a fuel, it is still usually produced via steam reforming of natural gas, petroleum hydrocarbon, nonrenewable materials and other hydrogenation reactions, which makes hydrogen production environmentally unfriendly (Ginkel and Sung, 2001). Biological process for hydrogen production is one of the alternative methods, can be operated at ambient temperatures, pressures, less energy intensive and more eco-friendly compared to conventional chemical method (Wang and Wan, 2009). This process is not only eco-friendly, but also escort to open new path for the exploitation of renewable energy resources which are unlimited. Biohydrogen production can be achieved by biophotolysis, photosynthesis /photo-fermentation, and dark fermentation. In a biophotolysis process, H_2O is “split” into H_2 and O_2 by green algae or cyanobacteria through two photosystems, and high intensity light is required to drive this process. With photo-synthesis/photo-fermentation, photosynthetic bacteria use a short-chain organic acid (e.g., acetate) as substrate to produce hydrogen through photosynthetic metabolism. Compared with biophotolysis, photo-fermentation is capable of using a wide spectrum of light, and it lacks oxygen evolving activity, which would otherwise cause oxygen inactivation. In addition, photosynthetic bacteria can utilize organic substrates from wastewater and the conversion yield is relative high. Photo-fermentation, however, has its own limitations (Kapdan and Kargi, 2006). Although oxygen inhibition is not as significant as in biophotolysis, it is still an inhibitor for the system. The process is also potentially limited by light availability.

Dark fermentation of sugar and organic waste materials presented a promising route of biohydrogen production because of its numerous advantages over other forms of hydrogen production. The major advantages of dark fermentative process are high rate of cell growth, operation without light source and no oxygen limitation problems (Levin et al., 2004). Various attempts have been made to generate fermentative hydrogen from biomass and wastewater like sugarcane bagasse (SCB) (Pattre et al., 2008), wheat straw (Chu, Y.

2011), cheese whey wastewater (Azbar et al., 2009) and dairy waste (Mohan et al., 2009). However, low hydrogen yields, low production rates and reactor instability for continuous high volume hydrogen production remain the major problems of the anaerobic method for hydrogen production at commercial levels. Another potential approach to enhance hydrogen production in anaerobic hydrogen production is to use an immobilized cell system (Hu and Chen, 2007). Immobilized cells offer distinct advantages over suspended cells, because they are resistant to cell wash-out during continuous operation and can maintain a higher cell density that increases hydrogen production. Immobilized cells have been successfully used for continuous biohydrogen production in a bioreactor (Chu, C.Y. 2011; Peixoto et al., 2011 and Keskin et al., 2011).

1.3 PROBLEM STATEMENT

Palm oil mill wastewater treatment systems are one of the major sources of green house gases in Malaysia due to their biogas emission (36% CH_4 with a flow rate of 5.4 l/min.m^2) from open digester tanks (Yacob et al., 2005). Also, the treated POME using ponding system sometimes could not meet the required discharge standards.

However anaerobic treatment of POME by UASB process is the most promising and useful technology due to the positive energy balance, inexpensive and high rate treatment system along with the production of usable biogas (Latif et al., 2011). Methane production through anaerobic digestion of POME is already broadly applied. Besides, methane has a relative lower calorific value (36.3 kJ/g), and CO_2 (22 g) is released during its combustion. In contrast, hydrogen gas has higher calorific value (118.2 kJ/g), and its reaction with oxygen does not produce green house gases such as CO_2. For example, to produce 1 kWh of eletricity need to burn ~ 30.5 g hydrogen where as to produce same amount of eletricity around ~ 90 g of methane (three times) must be burned. From this perspective, anaerobic bioconversion or organic wastes to hydrogen gas is an attractive option that achives both the goals.

Recently, the potential of using POME as the main substrate to produce biohydrogen has been revealed by severals group of researchers (Chong et al., 2009a; Chong et al., 2009b; Ismail et al., 2010 and Prasertsan et al., 2009). Up to now, biohydrogen production from POME in industrial scale is not ready based on current production technology. Apart from storage and safety problems of biohydrogen, problems associated with reactor design, long retention time and washout of bacteria with effluents may occur from the reactor at shorter HRTs of continuous dark fermentation have been identified as the major constrains to biohydrogen production. Another potential approach to conceptualize commercial scale of biohydrogen production is through cell immobilization. Bacteria immobilization enhances the available bacteria population in the reactor, increases fermentation rates, shortens the fermentation period, and increases the productivity. Recent studies show that different immobilization methods can be used as effective means to increase the hydrogen productivity with both pure and mixed cultures without washout of bacteria in the reactors at shorter HRTs (Chu, C.Y. 2011; Plangklang et al., 2012, and Zhao et al., 2012). This special feature clearly suggests that using immobilized cells might reduce the operational cost by gaining a comparable hydrogen producing capacity at a higher organic load rate (or lower HRTs). Polyethylene glycol (PEG) with additional merits was selected in this work for entrapment due to its simple immobilization procedure, low toxicity, good mechanical properties and highly porous structure that helps to sustain immobilized cell viability (Leenen et al., 1996). To the best of our knowledge, this is the first report of state of the art PEG-immobilized cell system for dark fermentative hydrogen production from POME.

1.4 RESEARCH OBJECTIVES

a) Main objective

The main objective of this study was to examine the enhanced biohydrogen production from POME using PEG-immobilized cells in a UASB reactor.

b) Specific Objectives

i. To determine the feasibility of PEG as a carrier to immobilize *Clostridium sp.* for hydrogen production from POME in batch test.

ii. To determine the hydrogen production performance of PEG-immobilized cell containing reactor in comparison to suspended cell reactor.

iii. To examine the application of a PEG-immobilized cell for continuous hydrogen production from POME in UASB reactor.

iv. To determine the effect of immobilized cell packing ratio, hydraulic time retention time and POME concentration on hydrogen production and treatment efficiency of POME.

v. To determine the optimized condition for hydrogen production capability of the immobilized cell, the conditions for cell immobilization including PEG concentration, cell loading, curing times as well as effects of temperature and inorganic components on hydrogen production.

1.5 SCOPE OF STUDY

To accomplish the above objectives, the following tasks were undertaken:

1. Raw POME was obtained from the final discharge point of a palm oil mill wastewater treatment plant, Lepar Hilir Pahang, Malaysia.
2. *Clostridium sp.* was obtained from Institute for Medical Research, Kuala Lumpur, Malaysia and used as an inoculum. Bacteria was acclimatized and stored in sterile 15% (v/v) glycerol solution at −10 ^{o}C before being subjected to immobilization.
3. Polyethylene glycol (PEG) gel was fabricated and used as a solid carrier to immobilize *Clostridium sp.*
4. Scanning electron microscopy (SEM) was used in order to examine the entrapped cells inside the PEG-immobilized bead.
5. The mechanical stability of the immobilized beads was expressed by the fracture frequency of the beads, f (%) = [N/Nt] × 100, where N is the number of fractured beads and Nt is the total number of beads.

6. Batch tests were carried out to study the effect of time 4 to 24 h on cell leakage from the bead into medium suspension and consequent effect on hydrogen production. Monod type kinetics model was used to study the effect of acclimated immobilized cells and unacclimated immobilized cell on hydrogen production.
7. The UASB reactor was designed by using stain less steel with a total volume of 5126 cm^3 and a 5-L working volume and used for continuous hydrogen production in this study.
8. Comparison of hydrogen production performance of immobilized cell reactor and suspended cell reactor at different HRT 2-12 h was also carried out.
9. The effect of packing ratio of immobilized cell, HRT, POME concentration on continuous hydrogen production and COD removal was carried out in UASB reactor.
10. Exploration and optimization of the hydrogen production capability of the immobilized cells as well as effect of temperature and addition of inorganic components on biohydrogen production was carried out.

1.6 THESIS ORGANIZATION

This thesis contained 5 chapters. In Chapter 1 (Introduction), a brief introduction of palm oil industry in Malaysia, palm oil mill effluent, Cell immobilization, UASB reactor, Biohydrogen production is described. Then a problem statement was given with some basis and rationale to find the directions and gaps in the study. Furthermore, research objectives of the present study are elaborated in detail together with the scope of the study to be covered. Additionally, the organization of thesis is also given in this chapter.

Chapter 2 (Literature Review) provide more detailed explanation of what has been summarized in Chapter 1. It also present reviews of published literatures, covering topics related to this study. Strength and shortcoming of some methods have also been discussed.

Chapter 3 (Materials and Methods) describes the materials and methods used in this research. A detail of experimental set-up is elaborated in this chapter. The last part of the chapter describes some important process control parameter calculations and analytical methods which have been used for experiment.

Chapter 4 (Results and Discussion) presents the findings obtained from the experiments carried out as in Chapter 3. A detailed discussion pertaining to the results obtained in this study and that from other studies will be provided.

Chapter 5 (Conclusions and Recommendations) concludes the findings from the current studies and recommendations for future studies in the related field made from the understanding and information generated in the present study. These recommendations are given due to their significance and importance to be further investigated and explored by future research work in this area.